Brit. J. vener. Dis. (1958), **34**, 246.

SENSITIVITY OF THE *TREPONEMA PALLIDUM* IMMOBILIZATION (TPI) TEST*†

A FUNCTION OF THE NUMBER OF SPIROCHAETES IN THE ANTIGEN

BY

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A major technical difficulty in the *Treponema pallidum* immobilization (TPI) test developed by Nelson and Mayer (1949) is the failure of their suspending medium to maintain consistently uniform survival of virulent *T. pallidum* employed as the antigen. For this reason, early studies to show that the sensitivity of the test may be increased by decreasing the concentration of spirochaetes resulted in failure (Nelson and Diesendruck, 1951). Portnoy, Olansky, and Edmundson (1953), employing a modified basal medium, observed an increase in the sensitivity of the test when the number of spirochaetes ranged from forty to fifteen per high dry dark-field. No alteration in sensitivity occurred, however, when the number of spirochaetes per high dry darkfield varied from fifteen to five.

The development of a suitable suspending medium by Boak and Miller (1954) led to further investigation of the problem. Evidence is hereby presented indicating that a proportionate relationship exists between the sensitivity of the TPI test and the number of spirochaetes when the antigen consists of from fifteen organisms to one organism per high dry darkfield.

Methods and Materials

The sensitivity and specificity of the TPI test were investigated employing one, five, and fifteen spirochaetes per high dry darkfield. The tests were carried out according to the method of Nelson and Mayer (1949), with the modifications described by Magnuson and Thompson (1949) and Boak and Miller (1954). The number of spirochaetes in the antigen was adjusted to contain fifteen spirochaetes per high dry darkfield, employing a Spencer binocular microscope equipped with 15× oculars and a 40× objective. The suspension was then diluted with the basal medium to provide antigens consisting of five to one spirochaete per field. The spirochaetes exhibited a 0-hour motility of 100 per cent. in each experiment.

The experimental procedure is presented in Table I (opposite).

A complete set of controls was included with each antigen. Anti-syphilitic rabbit sera were obtained from animals infected with the Nichols strain of virulent *T. pallidum*. Human sera were obtained from the Los Angeles City and County Health Departments, the Department of Infectious Diseases, University of California, Los Angeles, and from private physicians throughout the Los Angeles area. The recorded end-point of sera tested quantitatively was that point at which 50 per cent. of the spirochaetes were immobilized (Magnuson and Thompson, 1949).

Results

The 50 per cent. endpoint on 23 anti-syphilitic sera tested with antigen concentrations of fifteen, five, and one spirochaete per high dry field revealed changes in titre with the exception of one serum (Table II).

TABLE II

TITRES OBSERVED IN TPI TEST ON 23 ANTI-SYPHILITIC SERA*

Serum No.	Spirochaetes in Antigen per High Dry Darkfield		
	15	5	1
1	—	1:64	1:137
2	—	1:76	1:132
3	—	1:102	1:235
4	—	1:73	1:132
5	—	1:74	1:130
6	—	1:77	1:147
7	—	1:128	1:160
8	—	1:120	>1:160**
9	1:65	1:92	1:186
10	1:108	1:193	1:293
11	1:273	1:540	1:1051
12	1:52	1:97	1:173
13	1:67	1:210	1:387
14	1:36	1:49	1:100
15	1:109	1:135	1:131
16	1:16	1:28	1:35
17	1:17	1:25	1:47
18	1:18	1:29	1:51
19	1:60	1:113	1:258
20	1:17	1:30	1:53
21	1:143	1:225	1:470
22	1:120	1:300	1:617
23	1:126	1:143	1:160

* 50 per cent. immobilization designated as end-point.
** Further dilutions not completed.

* Presented before the Eighth Annual Symposium for the Recent Advances in the Study of Venereal Diseases, Washington, D.C., April, 1957.

† Received for publication June 26, 1958.

TABLE I

VARIATION IN SENSITIVITY OF TPI TEST

Count 0–1 Hour	Tube No.	Contents		Dilution	Motility of Spirochaetes		Per Cent.	Haemolysis	Per Cent. Difference	Result
		Serum	Complement		Active	Non-Motile				
	1	Normal	–		24	1	96	–		
	2	Normal	+		22	3	88	+	6	Negative
	3	Normal	+		23	2	92	+		
	4	Complement Control			23	2	92	+		
	5	Antigen Control			24	1	96	–		
15 Spirochaetes									50 per cent. End-point	
	6	Positive 1	–		22	3	88	–		
	7	Positive 1	+	1:10	1	24	8	+		
	8	Positive 1	+	1:20	2	23	8	+		
	9	Positive 1	+	1:40	10	15	40	+	1:65	
	10	Positive 1	+	1:80	14	11	56	+		
	11	Positive 1	+	1:160	19	6	76	+		
	1	Normal	–		22	3	88	–		
	2	Normal	+		24	1	96	+	0	Negative
	3	Normal	+		23	2	92	+		
	4	Complement Control			23	2	92	+		
	5	Antigen Control			20	5	80	–		
5 Spirochaetes									50 per cent. End-Point	
	6	Positive 1	–		23	2	92	–		
	7	Positive 1	+	1:20	1	24	4	+		
	8	Positive 1	+	1:40	5	20	20	+		
	9	Positive 1	+	1:80	11	14	44	+	1:92	
	10	Positive 1	+	1:160	21	4	84	+		
	11	Positive 1	+	1:320	22	3	88	+		
	1	Normal	–		21	4	84	–		
	2	Normal	+		23	2	92	+	0	Negative
	3	Normal	+		22	3	88	+		
	4	Complement Control			22	3	88	+		
	5	Antigen Control			21	4	84	–		
1 Spirochaete									50 per cent. End-Point	
	6	Positive 1	–		22	3	88	–		
	7	Positive 1	+	1:40	2	23	8	+		
	8	Positive 1	+	1:80	2	23	8	+		
	9	Positive 1	+	1:160	12	13	48	+	1:186	
	10	Positive 1	+	1:320	15	10	60	+		
	11	Positive 1	+	1:640	21	4	84	+		

Because of the complexities involved in the performance of a test utilizing a living antigen, however, alterations in sensitivity were considered significant only when two-fold dilution changes were observed. Significant increases in sensitivity were observed when the number of spirochaetes in the antigen was decreased from fifteen to five, from five to one, and from fifteen to one spirochaete per field (Table III).

TABLE III

EFFECT OF DECREASED NUMBERS OF *T. PALLIDUM* ANTIGEN ON SENSITIVITY OF TPI TEST AS MEASURED BY MINIMAL TWO-FOLD DILUTION CHANGE

No. of Spirochaetes in Antigen	Total Sera	Sera Exhibiting Altered Sensitivity	Sera Exhibiting Unaltered Sensitivity
15 and 5	15	13	2
5 and 1	23	20	3
15 and 1	15	14	1

Experiments were conducted to determine whether an alteration in the specificity of the test occurred by varying the number of spirochaetes in the antigen. Sera from 28 normal individuals and rabbits with non-reactive serologic tests for syphilis (STS) showed perfect correlation when tested with antigens consisting of five and one spirochaete per field. Similarly, sera from 47 patients with reactive STS and negative TPI tests when tested with five spirochaetes per field showed no reactivity when retested with the diluted antigen (Table IV). Thus, the specificity was not altered.

TABLE IV

EFFECT OF VARYING ANTIGEN CONCENTRATION ON SPECIFICITY OF TPI TEST

Result of TPI Test ..		Negative		Doubtful		Positive	
Number of Spirochaetes per High Dry Darkfield ..		5	1	5	1	5	1
Serum	Normal	28	28	0	0	0	0
	Biologic False Positive	47	47	0	0	0	0
	Total	75	75	0	0	0	0

After establishing that the sensitivity could be increased by decreasing the number of spirochaetes in the antigen, tests were performed using the diluted antigen on sixteen sera with doubtful TPI tests and on three with negative TPI tests which had previously been tested with five spirochaetes per field. Nine of the sera exhibiting a doubtful reaction became positive when the diluted antigen was used, and the tests on the negative sera resulted in doubtful reactions (Table V). Seven of the nine sera with the previous doubtful reactions which became positive, and the three which converted from negative to doubtful were obtained from patients with a history of primary syphilis and extensive therapy.

TABLE V

TPI TESTS ON NINETEEN SERA EMPLOYING ANTIGENS WITH FIVE AND WITH ONE SPIROCHAETE PER HIGH DRY DARKFIELD

Result of TPI Test	Spirochaetal Antigen Concentration	
	Results with 5 per Field	Altered Sensitivity with 1 per Field
Negative	3	3
Doubtful	16	9
Total	19	12

Discussion

The studies demonstrate that maximal sensitivity is not attained with a TPI antigen consisting of fifteen spirochaetes per high dry darkfield. The routine use of an antigen containing only one spirochaete per high dry darkfield is impractical, however, because of the difficulty in reading a test in which so few organisms are employed. An antigen containing five spirochaetes per field eliminates this shortcoming. Thus, TPI tests are routinely performed in this laboratory with approximately five spirochaetes per high dry darkfield to insure optimal sensitivity. If a "doubtful" TPI reaction is obtained with this antigen, the test may be repeated with an antigen containing only one spirochaete per field. A "negative" result with the diluted antigen indicates that the "doubtful" reaction was undoubtedly due to the presence of toxic substances in the serum rather than to the presence of immobilizing antibody. A "positive" result indicates the presence of immobilizing antibody. If, however, a "doubtful" reaction is obtained with the diluted antigen, a valid conclusion cannot be drawn because neither the possible presence of toxic substances nor the existence of immobilizing antibody can be excluded. Thus it was possible to establish a diagnosis of either present or past luetic infection in nine of sixteen patients, whereas previously the "doubtful" reaction was only presumptive evidence of the presence of antibody. The results further indicate that the sensitivity of the TPI tests varies inversely with the number of *T. pallidum* in the antigen.

Summary

(1) A method of increasing the sensitivity of the *Treponema pallidum* immobilization (TPI) test is described.

(2) Maximum sensitivity of the TPI test was obtained with an antigen containing one spirochaete per high dry darkfield.

(3) Inasmuch as the routine use of an antigen with only one spirochaete per high dry darkfield is impractical, an antigen containing five spirochaetes per high dry darkfield is recommended to insure optimal sensitivity in the TPI test.

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